DNA Repair and Cytokine Responses

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As sunscreens do not provide complete protection against solar/UV radiation, alternative protective strategies are necessary to cope with the increasing incidence of skin cancer. These strategies include the reduction of UVR-induced DNA damage by the topical application of bacterial DNA repair enzymes. Recent evidence suggests that nucleotide excision repair, the physiological repair system that is mostly responsible for the removal of UVR-mediated DNA damage, can be modulated by cytokines, including IL-12, IL-18, and α-melanocyte-stimulating hormone. The mechanisms involved and the biological as well as the potential therapeutic implications of these findings are discussed.

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INTRODUCTION

Ultraviolet radiation (UVR), in particular UVB, with a wavelength range between 290 and 320 nm, represents one, if not the most, important environmental factor among the inducible health hazards for mankind. These include the induction of skin cancer, suppression of the immune system, and premature skin aging. There is a substantial increase in the incidence of UVR-induced skin cancer, one of the most common malignancies in people of Caucasian descent, both in the United States and in Europe (Gloster and Brodland, 1996).

The basic event in photocarcinogenesis is the induction of DNA damage by UVR. UVB induces primarily two types of DNA lesions, cyclobutane pyrimidine dimers (CPD) and (6-4)-photoproducts. Induction of DNA lesions is a common event, occurring upon exposure to rather low, non-erythematogenic doses. The majority of these lesions are usually removed by nucleotide excision repair (NER), a complex repair process (de Laat *et al.*, 1999). The efficacy and the importance of NER are best shown by the disease xeroderma pigmentosum. Owing to genetic mutations in specific components of NER, xeroderma pigmentosum patients are severely impaired in their DNA repair capacity and, as a consequence, experience a dramatically (1,000-fold) increased incidence of skin cancer at early ages (Kraemer *et al.*, 1994).

However, there may be therapeutic hope for these patients. Other species, such as bacteria, also repair UVR-induced DNA damage, using other repair systems. Bacteria remove UVR-induced DNA damage using an enzyme called T4N5 endonuclease, marsupials using an enzyme called photolyase. Although not expressed in human cells, these repair enzymes repair equally well when delivered into human cells. A major breakthrough in this respect was achieved by the incorporation of these non-human repair enzymes into special liposomes, which enabled penetration into human cells and, even more importantly, into human skin when applied topically (Yarosh *et al.*, 1994). Accordingly, topical application of a T4N5 endonuclease lotion reduced the incidence of skin cancer in chronically UVR-exposed mice (Yarosh *et al.*, 1992). A multicenter double-blinded study in xeroderma pigmentosum patients revealed that application of a T4N5 endonuclease-containing lotion reduced significantly the incidence of actinic keratoses, the pre-stage of skin cancer, within a period of 1 year (Yarosh *et al.*, 2001).

Other DNA repair enzymes, including photolyase, have been shown to remove UVR-induced DNA damage on topical application, provided that they penetrate into the skin and into the cells (Stege *et al.*, 2000). These exogenous DNA repair enzymes are also suitable in helping remove UVR-induced DNA damage in normal individuals. This is of particular importance, as we have learned in recent years that conventional sun protection, even by potent sunscreens, is not fully effective. Therefore, any strategy supporting the removal of UVR-induced DNA damage after solar exposure should help to reduce the adverse effects of ambient solar radiation (Lautenschlager *et al.*, 2007).

Nature has taken care to provide humans with a system other than NER to protect against the consequences of UVR-induced DNA damage. If a cell is so severely damaged by UVR that it cannot remove the majority of DNA lesions, apoptosis is induced, thereby eliminating that cell (Brash *et al.*, 1996). These apoptotic keratinocytes, called sunburn cells, are frequently found in the UVR-exposed epidermis. Induction of UVR-mediated apoptosis seems to be regulated by the *p53* gene (Ziegler *et al.*, 1994). As *p53* eliminates DNA-damaged cells at risk for malignant transformation, it functions as a tumor suppressor gene. Taking this into account, the formation of sunburn cells may be regarded as

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Abbreviations: CPD, cyclobutane pyrimidine dimers; GTP, green tea phenol; α-MSH, α-melanocyte-stimulating hormone; NER, nucleotide excision repair

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a protective mechanism. Therefore, alterations or dysregulation in UVR-induced apoptosis may enhance the risk of developing skin cancer. Hence, whether UVR-induced apoptosis can be altered from the outside is a relevant question.

Cytokines can modulate UVR-induced apoptosis

It was observed that the cytokine, IL-1, enhances UVR-induced apoptosis, proving the hypothesis that cytokines can affect UVR-induced cell death (Kothny-Wilkes *et al*., 1999). As IL-1 is induced by UVR, it is tempting to speculate that this may represent an additional protection mechanism, by eliminating those cells that, although damaged, did not quite make it into apoptosis. On the basis of this observation, the effect of other cytokines on UVR-induced apoptosis was studied.

IL-12 is an immunomodulatory cytokine that plays an important role in the generation of Th1-driven immune responses (Trinchieri, 1993). In addition, IL-12 is able to reverse UVR-induced immunosuppression (Müller *et al*., 1995; Schmitt *et al*., 1995; Schwarz *et al*., 1996). It was more than surprising to observe that IL-12 significantly also reduces UVR-induced apoptosis *in vitro* (Schwarz *et al*., 2002). This was confirmed *in vivo*, as the injection of IL-12 into the skin of UVR-exposed mice significantly reduced the number of sunburn cells.

IL-12 affects DNA repair

At first glance, the above-mentioned discovery indicated IL-12 to be harmful in terms of photocarcinogenesis, as it inhibits UVR-induced apoptosis and thus may allow the survival of DNA-damaged cells. However, this was observed not to be the case during an attempt to elucidate the molecular mechanisms underlying this new biological activity of IL-12. When analyzing the amount of UVR-induced DNA damage, which is the major trigger for UVR-induced apoptosis (Kulms *et al*., 1999), it was noted that the amounts of CPD were reduced significantly in UVR-exposed cells treated with IL-12. The effect of IL-12 was not because of a filtering capacity, as the amounts of CPD were the same in the IL-12 and sham-treated groups, when DNA was extracted immediately after UVR exposure. This implied that the amount of DNA damage is initially the same; however, with increasing time, it decreases in the presence of IL-12. These surprising *in vitro* findings were also confirmed *in vivo*, as immunohistochemical analysis of the UVR-exposed murine skin revealed significantly reduced amounts of DNA damage *in situ* in mice that were injected intracutaneously with IL-12 before UVR exposure.

The fact that the amount of DNA damage was initially the same, but with time was reduced significantly in the presence of IL-12, inspired speculation that IL-12 facilitates the removal of UVR-induced DNA damage. As UVR-induced DNA damage is removed primarily by NER in human and murine cells, it was surmised that IL-12 might influence NER. To test this hypothesis, knockout mice, which were disrupted in the *Xpa* gene, were used. As the *Xpa* gene is a critical component of NER, these animals lack NER completely (de Vries *et al*., 1995). Intracutaneous injection of IL-12 into UVR-exposed wild-type mice significantly reduced the number of apoptotic keratinocytes, whereas IL-12 had no effect in the *Xpa* knockout mice (Schwarz *et al*., 2002). These findings indicated that IL-12 might inhibit UVR-induced apoptosis by reducing UVR-induced DNA damage, which ultimately might be attributed to the induction of NER. This was confirmed by an *in vivo* study. Upon chronic UVR exposure, IL-12-deficient mice developed skin tumors at a higher frequency when compared with wild-type mice (Maeda *et al*., 2006).

For some time, IL-12 has been known to be able to antagonize UVR-induced immunosuppression (Müller *et al*., 1995; Schmitt *et al*., 1995; Schwarz *et al*., 1996), although the mechanism remains to be elucidated. Currently, this activity also appears to be, at least partially, related to the effect of IL-12 on DNA repair, as IL-12 prevents both UVR-induced suppression during the induction of contact hypersensitivity and the depletion of Langerhans cells in wild-type but not in DNA repair-deficient mice (Schwarz *et al*., 2005). Thus, these findings identified a new mechanism by which IL-12 can protect immune responses and also have shown a link between DNA repair and the prevention of UVR-induced immunosuppression by IL-12 (Figure 1).

Although the underlying mechanisms are uncertain and remain to be elucidated, the observation that IL-12 influences NER might have important implications for several reasons. For a long time, it was thought that NER, as an essential repair system, is constitutively expressed and not subjected to any regulation. However, there are indications that NER, in contrast to this previous dogma, can be induced. Eller *et al*. (1997) first showed that administration of DNA oligonucleotides induces DNA repair through a *p53*-dependent mechanism. Accordingly, an *in vivo* study indicated that topical pretreatment with DNA oligonucleotides enhanced the rate of DNA photoproduct removal, decreased UVR-induced mutations, and reduced photocarcinogenesis in UVR-irradiated mice (Goukassian *et al*., 2001). Recently, it was observed this might also apply in humans, as treatment of skin explants obtained from adult human donors with T-oligos significantly reduced CPD upon UVR exposure

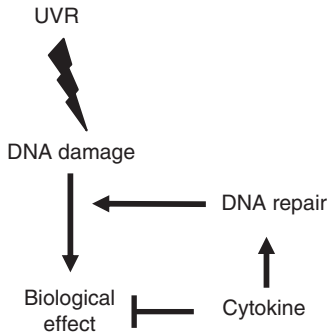

Figure 1. **Crosstalk between DNA damage, DNA repair, and cytokines.** UVR-induced DNA damage is a major molecular trigger for a variety of biological UVR effects, including the release of cytokines. By their ability to reduce DNA damage, presumably through the induction of DNA repair, various cytokines may inhibit or reduce some biological effects of UVR.

(Arad *et al*., 2006). This effect might have been mediated through *p53*, which was increased in T-oligo-pretreated skin compared with that in diluent-pretreated skin. The observations with IL-12 add to the concept that the NER can be induced by external stimuli.

The UVR protective effects of extracts from green tea phenols may be mediated through IL-12

IL-12 might also be involved in the recently described UVR protective effects of phenol extracts from green tea phenols (GTPs). GTPs protected against UVR-induced erythema, immunosuppression, sunburn cell formation, and skin cancer in animal models (Katiyar *et al*., 1995). Elmets *et al*. (2001) showed that GTPs exert similar effects in humans. When elucidating the underlying mechanism, Katiyar *et al*. (1999) showed that the prevention of UVB-induced immunosuppression in mice by GTPs may be associated with alterations in IL-10 and IL-12. Specifically, it was shown that application of GTPs before UVB exposure decreased the UVB-induced production of the immunosuppressive cytokine, IL-10, whereas the production of IL-12 was found to be markedly increased in the lymph nodes when compared with UVR-only-exposed mice (Katiyar *et al*., 1999).

Follow-up studies indicated that the prevention of UVR-induced immunosuppression by GTPs in mice is mediated through IL-12-dependent DNA repair (Meeran *et al*., 2006b). Topical application of GTPs prevented UVR-induced suppression of contact hypersensitivity in wild-type but not in IL-12-deficient mice. Accordingly, injection of an anti-IL-12 antibody blocked the preventive effect of GTPs on UVR-induced immunosuppression. In addition, GTP-induced reduction of CPD was not observed in IL-12-deficient mice. The link between GTPs and NER was confirmed by the observation that GTPs did not remove CPD in NER-deficient cells (Meeran *et al*., 2006a). Furthermore, GTPs were shown to prevent photocarcinogenesis by IL-12-dependent DNA repair. GTPs did not prevent the induction of skin cancer by UVR in IL-12 knockout mice. Accordingly, CPD and sunburn cells resolved more rapidly in wild-type mice upon application of GTPs, but not in IL-12-deficient mice (Meeran *et al*., 2006a).

IL-18 reduces UVR-induced DNA damage

The capacity to reduce UVR-induced DNA damage, and probably to affect DNA repair, does not appear to be specific to IL-12. The proinflammatory cytokine, IL-18, seems to exert similar effects (Schwarz *et al*., 2006). Injection of IL-18 into UVR-exposed skin reduced the amount of DNA damage in normal but not in NER-deficient mice, indicating that IL-18 like IL-12 reduces DNA damage by DNA repair. UVR-mediated suppression of the induction of contact hypersensitivity was prevented upon injection of IL-18 before UVR exposure in wild-type mice but not in NER-deficient mice. In contrast to IL-12, IL-18 was not able, either in wild-type or in NER-deficient mice, to break UVR-induced immunotolerance, which is mediated through regulatory T cells, but independent of DNA damage (Beissert *et al*., 2006). This indicates that, although being primarily a proinflammatory cytokine through an effect on DNA damage, IL-18 can also foster an immune response that is suppressed by UVR (Figure 2). However, IL-12 is still unique in its capacity to restore immune responses because of its effect on regulatory T cells.

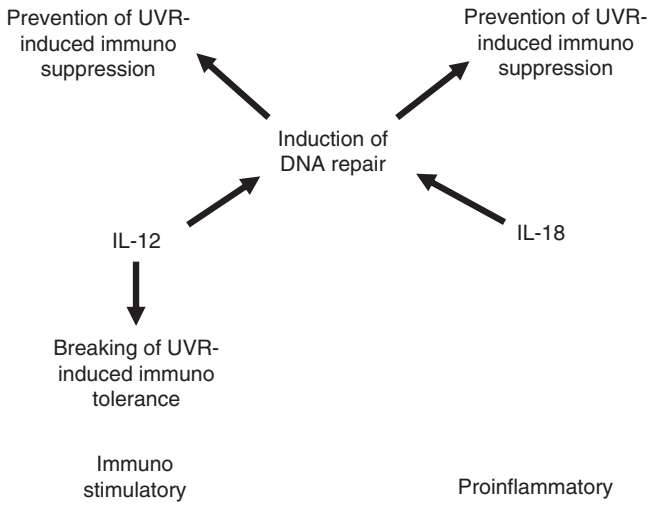

Figure 2. **Effects of IL-12 and IL-18 on UVR-induced immunosuppression.** Both IL-12 and IL-18 prevent UVR-induced immunosuppression through the modulation of DNA repair. Accordingly, suppression of the induction of contact hypersensitivity by UVR, which is mediated by DNA damage, is not observed upon injection of either IL-12 or IL-18. In contrast, established UVR-mediated immunotolerance can only be broken by IL-12 but not by IL-18, indicating that IL-12 exerts more pronounced immunostimulatory features than IL-18. Although being primarily a proinflammatory cytokine, IL-18 prevents UVR-induced immunosuppression by its capacity to induce DNA repair. (Adapted with permission from Schwarz T, *Photochem Photobiol* 2008, **84**: 10–18).

The neuropeptide α-melanocyte-stimulating hormone reduces UVR-induced DNA damage

The neuropeptide, α-melanocyte-stimulating hormone (α-MSH), was found to block UVR-induced apoptosis by normal human melanocytes *in vitro* (Böhm *et al*., 2005). The anti-apoptotic activity of α-MSH was not mediated by a filtering effect or by the induction of melanin synthesis in melanocytes. α-MSH influenced neither the expression of the apoptosis-related proteins (Bcl_2, Bcl_x, p53, Fas, and FasL) nor the cell cycle. However, α-MSH reduced the amount of UVB-induced DNA damage remarkably as shown by Southwestern blot analysis. The reduction of UVR-induced DNA damage by α-MSH may also be related to NER, as UVR-mediated apoptosis was not blocked by α-MSH in NER-deficient fibroblasts. These data implicate the regulation of UVR-induced apoptosis of human melanocytes by a neuropeptide, which is physiologically expressed within the epidermis. Furthermore, this suggests that besides its ability to induce photoprotective melanin synthesis, α-MSH may exhibit a capacity to reduce UVR-induced DNA damage. Thus, it is tempting to speculate that through this activity α-MSH may act as a protection factor against the harmful effects of UVR on the genomic stability of epidermal cells.

CONCLUSION

UVR-induced DNA damage is regarded as the major trigger for most of the biological effects of UVR. Consequently, this signal transduction pathway was supposed to be unidirectional, as any biological effect should be the consequence of DNA damage. The observation that several cytokines can, in turn, control DNA repair and consequently UVR-induced DNA damage suggests that this signaling pathway may not be as unidirectional as initially anticipated, but indicates the existence of a biofeedback mechanism (Figure 1). This crosstalk may represent a new defense mechanism of the host against UVR-induced immunosuppression and of carcinogenesis. Future studies will clarify whether this observation is of practical relevance in the development of sun protection strategies.

CONFLICT OF INTEREST

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